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INTRACELLULAR pH AND ITS REGULATION IN ISOLATED TYPE I CAROTID BODY CELLS OF THE NEONATAL RAT

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SUMMARY

1. The dual-emission pH-sensitive fluoroprobe carboxy-SNARF-1 (carboxy-seminaptharhodofluor) was used to measure pH_i in type I cells enzymically dispersed from the neonatal rat carotid body.

2. Steady-state pH_i in cells bathed in a HEPES-buffered Tyrode solution (pH 7·4) was found to be remarkably alkaline ($pH_i = 7·77$) whereas cells bathed in a CO_2–HCO_3^--buffered Tyrode solution (pH 7·4) had a more 'normal' pH_i ($pH_i = 7·28$). These observations were further substantiated by using an independent null-point test method to determine pH_i.

3. Intracellular intrinsic buffering (β, determined by acidifying the cell using an NH_4Cl pre-pulse) was in the range 7–20 mM per pH unit and appeared to be dependent upon pH_i with β increasing as pH_i decreased.

4. In cells bathed in a HEPES-buffered Tyrode solution, pH_i recovery from an induced intracellular acid load (10 mM-NH_4Cl pre-pulse) was inhibited by the Na^+–H^+ exchange inhibitor ethyl isopropyl amiloride (EIPA; 150 μM) or substitution of Na_o^+ with *N*-methyl-D-glucamine (NMG). Both EIPA and Na_o^+ removal also caused a rapid intracellular acidification, which in the case of Na_o^+ removal, was readily reversible. The rate of this acidification was similar for both Na_o^+ removal and EIPA addition.

5. Transfering cells from a HEPES-buffered Tyrode solution to one buffered with 5% CO_2–HCO_3^- resulted in an intracellular acidification which was partially, or wholly, sustained. The rate of acidification upon transfer to CO_2–HCO_3^- was considerably slowed by the membrane permeant carbonic anhydrase inhibitor, acetazolamide, thus indicating the presence of the enzyme in these cells.

6. In CO_2–HCO_3^--buffered Tyrode solution, pH_i recovery from an intracellular acidosis (NH_4^+ pre-pulse) was only partially inhibited by EIPA or amiloride whereas Na_o^+ removal completely inhibited the recovery. The stilbene DIDS (4,4-diisothiocyanatostilbenedisulphonic acid, 200 μM) also partially inhibited pH_i recovery following an induced intracellular acidosis. Furthermore, the pre-treatment with 200 μM-DIDS of a pre-acidified cell in Na^+-free Tyrode solution completely inhibited pH_i recovery when Na_o^+ was reintroduced together with concomitant addition of

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150 μM-EIPA. We conclude, that in the presence of CO_2–HCO_3^-, a Na^+- and HCO_3^--dependent (DIDS inhibitable) mechanism aids acid extrusion.

7. The application of DIDS to cells in CO_2–HCO_3^--buffered Tyrode solution was frequently observed to cause a small alkalosis ($\approx$ 0·1 pH units) suggesting that DIDS may also inhibit a mechanism which mediates a background acid influx in the steady state.

8. Removal of external Cl^- (glucuronate and gluconate substituted) in CO_2–HCO_3^--buffered Tyrode solution caused a large ($\approx$ 0·5 pH units) reversible intracellular alkalosis. This alkalosis was inhibited by the anion exchange inhibitor DIDS but not by removal of external Na^+ (NMG substituted).

9. We conclude that the type I cells from neonatal rat carotid bodies possess at least three mechanisms for transmembrane acid equivalent transport: (i) Na^+–H^+ exchange which extrudes acid from the cell, (ii) another acid extrusion mechanism which requires both external Na^+ and bicarbonate and which is DIDS inhibitable, and (iii) a Na^+-independent Cl^-–HCO_3^- exchange which probably mediates a background acid influx.

INTRODUCTION

The carotid body is the principal O_2 and pH chemoreceptor for arterial blood. It is excited by hypoxia or acidosis and initiates corrective reflex changes in ventilation. Several lines of evidence suggest that the catecholamine-containing type I cells (which are arranged in glomi surrounding afferent sensory fibres) are the chemosensory elements of this organ (see Fidone & Gonzalez, 1986, for review). The function of the glial-like type II cells is less well defined. Recent studies of isolated type I cells using patch-clamp techniques and Ca_i^{2+} microfluorimetry have furnished much new information relating to O_2 detection (Biscoe & Duchen, 1989; Hescheler, Delpiano, Acker & Pietruschka, 1989; Lopez-Lopez, Gonzalez, Urena & Lopez-Barneo, 1989). The mechanism, however, for H^+ ion detection remains unknown. Certain classes of K^+ channel in the type I cell are known to be blocked by reducing extracellular pH (Lopez-Lopez *et al.* 1989; Peers, 1990), but whether this effect is physiologically important remains to be established. Furthermore, it is not known if the relevant H^+ reception site is intracellular or extracellular. Current consensus favours an intracellular site, in which case pH_i could be an important variable governing chemotransduction. At present there is little information concerning pH_i in the type I cell. A recent report (He, Wei & Eyzaguirre, 1990) suggests that pH_i (measured with an ion-selective microelectrode) is particularly low (6·8) but this has yet to be confirmed. Furthermore the possibility of regulation of intracellular pH has not so far been investigated.

We have developed the use of the novel single-excitation, dual-emission fluoroprobe carboxy-SNARF-1 (carboxy-seminaptharhodofluor) for measurement of pH_i in single type I cells isolated from the neonatal rat (Buckler & Vaughan-Jones, 1990). We now report that pH_i in these cells is regulated efficiently by means of at least three ionic mechanisms that move acid (or its ionic equivalent) across the surface membrane. We find that pH_i is critically dependent upon the presence of CO_2. If a HEPES-buffered (CO_2 free) perfusate is employed, pH_i is variable and can be rather alkaline (7·8). In 5% CO_2–HCO_3^--buffered media however (a more

physiological condition), pH_i is about 7·3 and varies little among cells. We consider possible reasons for the variability of pH_i in the absence of CO_2 and we discuss the functional importance of pH_i regulation in the type I cell.

A preliminary account of this work has appeared (Buckler, Nye, Peers & Vaughan-Jones, 1990*a*).

METHODS

Cell isolation

Type I cells were isolated using the method described by Peers & O'Donnell (1990). In brief, carotid bodies were removed from 7- to 11-day-old rat pups anaesthetized by breathing 4% enflurane in O_2 through a face mask. The carotid bodies were dissociated by incubation in phosphate-buffered saline containing 0·03–0·05% collagenase and 0·020–0·025% trypsin for 30 min at 37 °C, followed by gentle trituration. This medium was then exchanged for culture medium (usually HCO_3^--buffered Ham's F-12, but for some studies Dulbecco's modified Eagle's medium (DMEM) or HEPES-buffered Ham's F-12 media were used). All media contained insulin (84 U l^{-1}), penicillin (100 i.u. ml^{-1}) streptomycin (100 μg ml^{-1}) and 10% fetal calf serum. The dispersed cells were then placed onto poly-L-lysine-coated cover-slips and kept in culture for between 4 and 40 h before being used for microfluorimetric studies. Single type I cells were selected for study as previously described (Peers & O'Donnell, 1990); recordings were only made from the most commonly found spherical, phase-bright cells of approximately 10 μm diameter, or clusters of such cells. An additional piece of evidence that these were indeed the type I cells was that the membrane permeant carbonic anhydrase inhibitor, acetazolamide, considerably reduced the rate at which an elevated P_{CO_2} could acidify pH_i (see Fig. 9 and Results), consistent with an intracellular presence of the enzyme as anticipated from its previous identification within type I cells (Ridderstråle & Hanson, 1984; Rigual, Iniquez, Carreres & Gonzalez, 1985).

Solutions

HEPES-buffered Tyrode solution contained (in mM): NaCl, 140; KCl, 4·5; $MgCl_2$, 1; $CaCl_2$, 2·5; glucose, 11; HEPES, 20; pH was adjusted to 7·4 at 37 °C with NaOH. Sodium-free HEPES Tyrode solution contained (in place of NaCl) 140 mM-*N*-methyl-D-glucamine (NMG); the pH was adjusted to 7·4 with HCl. Bicarbonate-buffered Tyrode solution contained (in mM): NaCl, 117; KCl, 4·5; $NaHCO_3$, 23; $MgCl_2$, 1·0; $CaCl_2$, 2·5; glucose, 11; and was equilibrated with 5% CO_2–95% O_2; pH at 37 °C was 7·4–7·45. Sodium-free CO_2–HCO_3^--buffered Tyrode solution contained 140 mM-NMG (in place of NaCl and $NaHCO_3$); pH was adjusted to 7·4 with HCl while continuously bubbling with 5% CO_2–95% O_2. Chloride-free CO_2–HCO_3^--buffered Tyrode solution contained (in mM): sodium glucuronate, 117; potassium gluconate, 4·5; calcium gluconate, 12 (Ca^{2+} was elevated to compensate for its binding to gluconate and glucuronate, cf. Vaughan-Jones, 1979*a*); $NaHCO_3$, 23; $MgSO_4$, 1; glucose, 11. Sodium- and chloride-free CO_2–HCO_3^--buffered Tyrode solution were as for Cl-free Tyrode solution but with sodium glucuronate and $NaHCO_3$ replaced by 140 mM-NMG and 117 mM-glucuronic acid; following equilibration with 5% CO_2–95% O_2, pH was further adjusted if necessary by the addition of solid NMG or glucuronic acid. Nigericin calibration solutions contained (in mM): KCl, 140; $MgCl_2$, 1·0; nigericin, 10 μM; buffered with one of the following organic buffers (Sigma) 20 mM-MES(2-(*N*-morpholino)ethanesulphonic acid; pH = 5·5), 10 mM-PIPES(piperazine-*N*-*N*′-bis(2-ethanesulphonic acid)) + 10 mM-HEPES (pH = 6·5, 7·0, 7·5, 8·0) or 20 mM-CAPSO (3-cyclohexylamino-2-hydroxy-1-propanesulphonic acid; pH = 9·5) and was adjusted to the desired pH at 37 °C with NaOH.

Null-point test solutions (NPT; Eisner, Kenning, O'Neil, Pocock, Richards & Valdeolmillos, 1989) contained the following concentrations of trimethylamine chloride (TMA-Cl) and sodium propionate: (i) NPT 7·1, 4 mM-TMA-Cl and 16 mM-sodium propionate; (ii) NPT 7·2, 6 mM-TMA-Cl and 15 mM-sodium propionate; (iii) NPT 7·4, 6 mM-TMA-Cl and 6 mM-sodium propionate; (iv) NPT 7·6, 4 mM-TMA-Cl and 1·6 mM-sodium propionate; (v) NPT 7·8, 4 mM-TMA-Cl and 0·06 mM-sodium propionate.

Drugs

The following drugs were used: 4,4′-diisothiocyanatostilbene-2,2′-disulphonic acid (DIDS), amiloride (both Sigma) and ethyl isopropyl amiloride (EIPA, generous gift of W. Fuhrer and K. Scheibli, Ciba-Geigy, Basel, Switzerland).

Loading of carboxy-SNARF-1 into cells and its calibration

Cells were loaded with carboxy-SNARF-1 by incubating them in a 5–10 μM solution of the acetoxymethyl ester (AM form) in HEPES Tyrode solution for 8 min at room temperature. At the end of the experiment, cells were superfused with nigericin plus high K^+ calibration solutions of pH 5·5–9·5 (see below). This technique utilizes the ability of nigericin to equilibrate internal and external pH (Thomas, Buchsbaum, Zimniak & Racker, 1979).

Microspectrofluorimetry

An inverted microscope (Nikon Diaphot) was converted for epifluorescence. A carboxy-SNARF-1-loaded cell in an enclosed experimental chamber was placed on the microscope stage and excited with light at 540 ± 12 nm. The fluorescence emitted was measured simultaneously at 590 ± 5 and 640 ± 5 nm using two photomultiplier tubes. The outputs from the photomultiplier tubes were fed to an *I–V* converter and filtered at 10 Hz (3 dB). The signals were then digitized at 0·5 kHz (Cambridge Electronic Design, CED 1401 intelligent interface) and stored for later analysis on the hard disc of a microcomputer (Tandon PCA40+). Typically, values were averaged over 2 s periods and then digitally filtered by a 3-point rolling average. Where very rapid pH changes were of interest (e.g. Fig. 9) the signals were averaged over 0·5 s and further filtering was omitted.

The emission ratio of 590/640 was calculated and converted to a linear pH scale by using the following equation (see Grynkiewicz, Poenie & Tsien, 1985):

$$\mathrm{pH} = \mathrm{p}K_a - \log\frac{R - R_{min}}{R_{max} - R} + \log F_{640_{min/max}}, \quad (1)$$

where R is the fluorescence emission ratio 590/640 nm, R_{max} is the emission ratio at low pH, i.e. when the fluoroprobe is predominantly in the protonated form, R_{min} is the emission ratio at high pH, i.e. when the fluoroprobe is predominantly in the non-protonated form, and $F_{640_{min/max}}$ is the ratio of fluorescence measured at 640 nm for low pH to that for high pH. The values of the constants R_{min}, R_{max} and $F_{640_{min/max}}$ were determined using nigericin calibration solutions of pH 5·5 and 9·5. pK_a was determined from the value of R obtained in an intermediate calibration solution (e.g. pH 7·5) using the above constants and eqn (1). In most cases these constants were determined for each individual cell but, in some, the averaged values obtained from several previous experiments were used (e.g. Fig. 4*A*). For further details of the experimental apparatus and techniques see Buckler & Vaughan-Jones (1990).

Estimation of intracellular, intrinsic buffering power

Intrinsic buffering power (β, mM (pH unit)$^{-1}$) was estimated from the calculation of intracellular acid generated following removal of external NH_4^+ (ammonium pre-pulse technique, see Roos & Boron, 1981) and the resulting pH_i change measured under nominally CO_2–HCO_3^--free conditions and under conditions which inhibit acid extrusion (Bountra, Powell & Vaughan-Jones, 1990). The amount of intracellular acid generated by this technique was assumed to be equal to the intracellular NH_4^+ concentration immediately prior to its removal from the extracellular medium, i.e. all intracellular NH_4^+ was assumed to diffuse out of the cell as NH_3 leaving behind an equimolar amount of H^+. Intracellular NH_4^+ was calculated as follows, $[NH_4^+]_i = [NH_4^+]_o\ [H^+]_i/[H^+]_o$. ($[NH_4^+]_o$ was calculated from the Henderson–Hasselbalch equation assuming a pK for NH_4Cl of 9·02; see Vaughan-Jones & Wu, 1990, for further details.) Intracellular buffering power was then calculated as $\beta = [NH_4^+]_i/\Delta pH_i$.

Analyses of net acid efflux

Net acid efflux (J_H) was estimated using the equation

$$J_H = \beta\, \mathrm{d}(pH_i)/\mathrm{d}t, \quad (2)$$

$$\text{where} \quad \beta = 101{\cdot}3 - 12{\cdot}4\ (pH_i). \quad (3)$$

Equation (2) is standard for determination of acid efflux from pH_i recovery rate (Roos & Boron, 1981). The pH_i recovery rate was estimated as the gradient for successive pH_i changes (measured from calibrated recordings of pH_i) determined at 5–30 s time intervals. Each value of $d(pH_i)/dt$ was referred to the mean value of pH_i for that particular gradient determination. Equation (3) was determined empirically for the carotid body type I cell using the line of best fit to the data shown in Fig. 7.

RESULTS

Intracellular pH in the type I cell

Figure 1 illustrates the use of SNARF-1 to record pH_i in a type I cell perfused with HEPES-buffered solution. The top two traces show fluorescent emission intensity recorded at 590 ± 5 and 640 ± 5 nm. Note that, at these wavelengths, changes of pH produce opposite changes in fluorescence. The ratiometric recording of pH_i computed using these two traces is shown in the recording at the bottom of the figure. This latter trace was calibrated in pH units by reference to the *in situ* nigericin calibration performed at the end of the experiment (see Methods for further details). The estimated value of intracellular pH was somewhat higher than that found typically in other cell types. The value of pH_i recorded in twenty-two HEPES-perfused type I cells varied from 7·5 to 8·0 (mean $pH_i \pm$ S.E.M., $7{\cdot}77 \pm 0{\cdot}04$).

Figure 1 shows that, following an intracellular acidosis induced by addition and then removal of extracellular NH_4Cl (10 mM), pH_i returned within 5 min to the control level. The response of pH_i to NH_4Cl was therefore qualitatively similar to that observed in other cell types (i.e. a transient intracellular alkalosis upon NH_4Cl addition and an acidosis upon its removal, followed by pH_i recovery, cf. Thomas, 1984). Thus, the type I cell is capable of regulating pH_i following intracellular acidosis.

Figure 2 shows the effect on pH_i of switching from HEPES-buffered solution to one buffered (same pH_o of 7·4) using 5% CO_2–23 mM-HCO_3^-. The initial pH_i of the two cells shown was different (8·0 and 7·65) but both rapidly acidified in CO_2–HCO_3^--buffered Tyrode solution, presumably reflecting entry and subsequent hydration of CO_2. The interesting point is that, in the steady state, pH_i in both cells was now virtually identical at 7·3–7·35. This was typical of all our results, pH_i in CO_2–HCO_3^- media was $7{\cdot}28 \pm 0{\cdot}01$ (S.E.M.; $n = 22$), less alkaline and far less variable than pH_i observed in HEPES media. We also found that the dynamic response of pH_i to application of CO_2–HCO_3^- was variable among cells even though the *final* pH_i was usually the same. Two types of dynamic response were typically observed and these correlated with the initial value of pH_i seen in HEPES solution. When the initial pH_i was rather alkaline, the response was as in Fig. 2*A*, a rapid monotonic fall in pH_i (see also Fig. 9), but when initial pH_i was less alkaline the response was as in Fig. 2*B*, a transient overshoot of pH_i (see also beginning of Fig. 13).

Thus, in CO_2–HCO_3^- solution, pH_i in the type I cell is more homogeneous and more acid, closer to the resting value of pH_i seen typically in other cells (i.e. 7·2–7·3).

Does carboxy-SNARF-1 give a true record of pH_i?

Three pieces of evidence suggest that the alkaline pH_i in HEPES solution is real and not an artifact of the fluoroprobe recording or its calibration.

Firstly, any systematic error in calibration would affect pH_i measurement in both HEPES *and* CO_2–HCO_3^--buffered media.

Secondly, in some experiments, the value of pH_i was confirmed using a *null-point* method. This is shown in Fig. 3. The null-point method (Eisner *et al.* 1989) is based

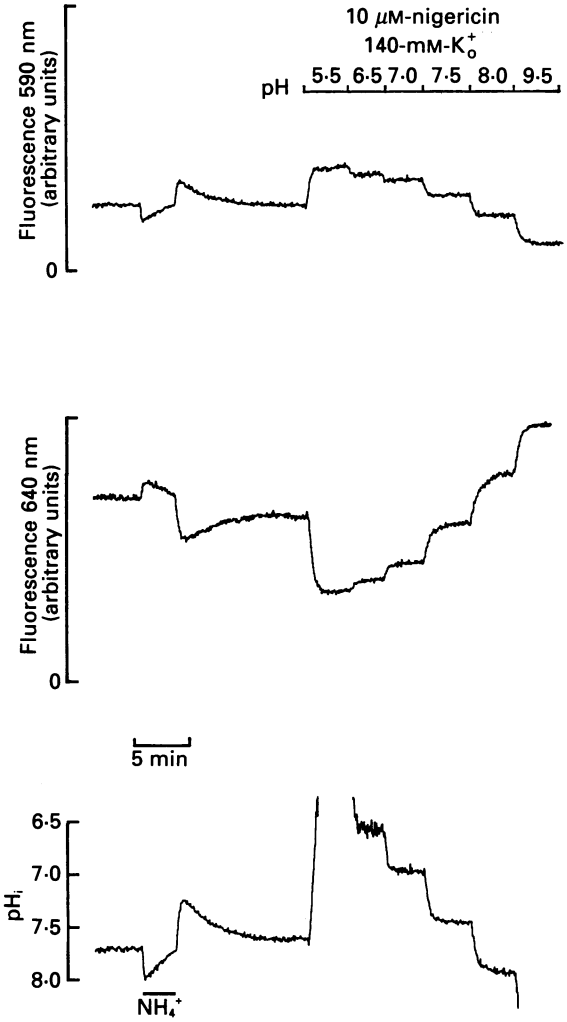

Fig. 1. Recording of pH_i in a single type I carotid body cell, using carboxy-SNARF-1 (loaded in AM form, see Methods). The top and middle traces show the intensity of fluorescence at 590 and 640 nm respectively. The bottom trace shows a fully calibrated (linearized) recording of the ratio of fluorescence 590/640. The first part of the recordings (i.e. addition and removal of 10 mM-NH_4Cl as indicated by bar) was obtained while bathing the cell in HEPES-buffered Tyrode solution, the later part (i.e. the calibration procedure) was performed in nigericin calibration solutions (in which pH_i is assumed to equal pH_o) of various pH values (see Methods for further details).

on the fact that for a given value of pH_i simultaneously applying an external permeant weak acid (propionic acid) plus a permeant weak base (trimethylamine) in a particular ratio will induce *no* change of pH_i. This ratio is related uniquely to a

single pH_i value. If pH_i is lower than this value, then it will *increase* upon acid/base application and vice versa. Figure 3*A* shows a recording of pH_i from a cell in HEPES-buffered solution. Two different acid/base ratios were applied, designed to equilibrate at pH_i 7·8 and 7·6 respectively. In the first case (null point (NP) = 7·8),

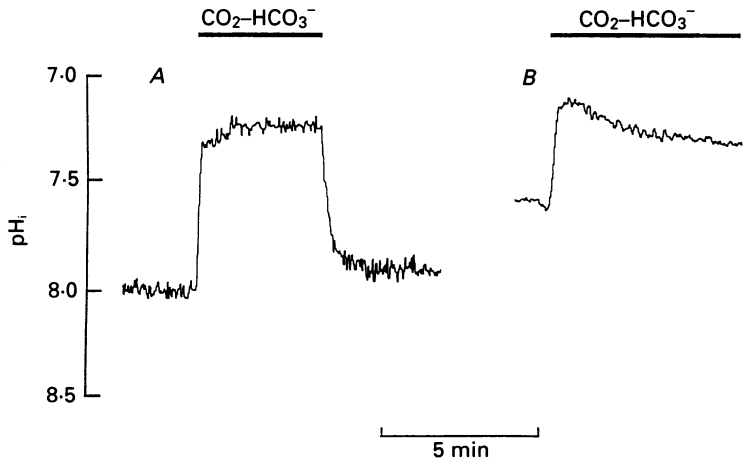

Fig. 2. Effects on type I carotid body cell pH_i of replacing a HEPES-buffered Tyrode solution (pH_o 7·4) with a 5% CO_2–23 mM-HCO_3^--buffered Tyrode solution of the same pH. *A* and *B* are the responses of two different cells with differing initial pH_i in HEPES-buffered Tyrode solution.

pH_i increased and in the second case (NP = 7·6), it *decreased*. The real value of pH_i was thus between 7·6 and 7·8, a value close to that estimated in the same cell using the nigericin calibration (pH_i = 7·80). In three such experiments the mean value for pH_i was found to be 7·7 by the nigericin calibration and 7·63 using the null-point test method. Thus two independent estimates of pH_i indicated an alkaline value. In contrast, Fig. 3*B* shows that the null-point test and nigericin calibration both indicated a more acidic pH_i (7·2) in CO_2–HCO_3^--buffered solution (in ten experiments mean $pH_i \pm$ S.E.M. was found to be $7{\cdot}23 \pm 0{\cdot}04$ by the nigericin calibration technique and $7{\cdot}18 \pm 0{\cdot}05$ by the null-point test). It is thus unlikely that the difference of pH_i recorded from cells in HEPES and CO_2–HCO_3^--buffered media is an artifact of the recording system.

A third reason for believing the alkaline pH_i reading in type I cells bathed in HEPES media is that it depended upon the conditions used for overnight cell storage following enzymic isolation. Figure 4*A* illustrates an experiment where type I cells were stored at 37 °C in 5% CO_2–HCO_3^--buffered Ham's medium (as usual) and were then switched to HEPES Tyrode solution a few minutes before recording pH_i. Under these conditions, pH_i was found to be alkaline ($pH_i = 7{\cdot}74 \pm 0{\cdot}08$; $n = 20$) as before. Figure 4*B* shows that in other cells from the same preparation, but kept overnight at 37 °C in HEPES-buffered Ham's medium, pH_i in HEPES Tyrode solution was much closer to 'normal' values of pH_i i.e. $7{\cdot}3 \pm 0{\cdot}04$ ($n = 21$). Furthermore, in these latter cells switching to a CO_2–HCO_3^- solution now produced a transient acidosis (Fig. 4*C*). The steady-state pH_i difference between HEPES and CO_2–HCO_3^- media

was almost entirely removed. Possible reasons for this are considered in the Discussion. Suffice it to say here that the pH_i difference, when it occurs, is a function of initial storage/culture conditions rather than an artifact of SNARF-1 fluorescence. Note also that pH_i with CO_2–HCO_3^- solution is *independent* of initial storage/culture conditions and is stable at $\approx 7{\cdot}20$.

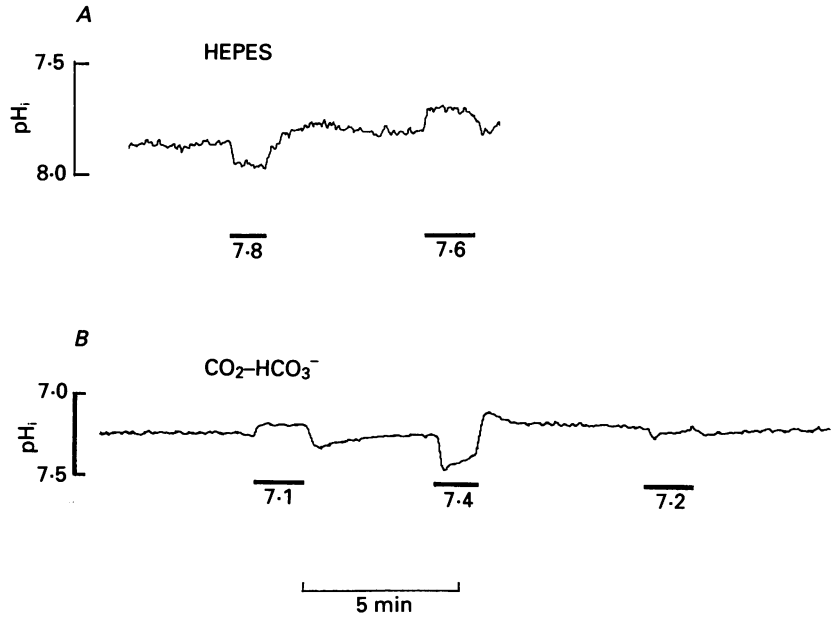

Fig. 3. Determination of intracellular pH using the null-point method in two different cells. *A*, cell bathed in HEPES-buffered Tyrode solution (pH 7·4). *B*, cell bathed in 5% CO_2–HCO_3^--buffered Tyrode solution (pH 7·4); taken from Fig. 2 of Buckler & Vaughan-Jones (1990) with permission. Ordinate, pH_i determined from nigericin calibration of fluorescence ratio. Horizontal bars refer to application of null-point test solutions. These are mixtures of a weak acid [AH] with a weak base [B]. In their un-ionized form both acid and base are permeant and will therefore enter the cell until the following equilibrium conditions are met:

$$K_a[A^-]_o[H^+]_o = [AH]_o = [AH]_i = K_a[A^-]_i[H^+]_i \quad \text{and}$$

$$K_b[BH^+]_o/[H^+]_o = [B]_o = [B]_i = K_b[BH^+]_i/[H^+]_i.$$

For any given ratio of external weak acid to weak base there is a unique value of pH_i for which there is no net change in pH_i i.e. $[A^-]_i = [BH^+]_i$. This value is indicated below each bar, the concentrations of weak acid and base for each solution are given in Methods. For comparison the pH_i scale to left of recordings was obtained using the standard nigericin (+high K^+) calibration procedure (see Methods).

pH_i recovery in CO_2-free conditions: Na^+–H^+ exchange

Figure 5*A* shows the effect on pH_i of removing and re-adding external Na^+ at two different values of pH_i. In the first part of the experiment a brief period of Na_o^+-removal (Na_o^+ replaced with *N*-methyl-D-glucamine) produced a steady fall of pH_i

from 7·9 to 7·6 that was reversed by re-admitting Na^+. The pH_i was then acidified further (to 7·15) using the NH_4Cl pre-pulse technique, while simultaneously removing Na_o^+. Note that there was no pH_i recovery (pH_i continued to fall slowly) until Na_o^+ was re-admitted. Figure 5*B* shows that this Na_o^+-activated pH_i recovery was

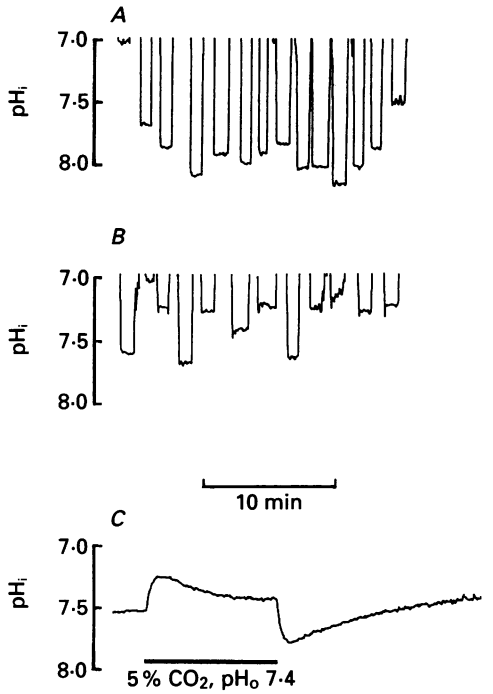

Fig. 4. *A* and *B*, sequential recordings of pH_i from different type I carotid body cells in HEPES-buffered Tyrode solution. *A*, measurements from fourteen cells kept in 5% CO_2–HCO_3^--buffered Ham's F-12 medium overnight; *B*, representative recordings from twelve cells kept in HEPES-buffered Ham's F-12 medium overnight. *C*, pH_i recorded in a single cell kept in HEPES-buffered Ham's F-12 overnight, cell initially in HEPES-buffered Tyrode solution, pH 7·4, then transferred to a 5% CO_2–HCO_3^--buffered Tyrode solution, pH 7·4, for period indicated.

inhibited by a high dose (100 μM) of EIPA, a high-affinity Na^+–H^+ exchange inhibitor (Kleyman & Cragoe, 1988). An NH_4^+ pre-pulse in the presence of EIPA produced a sustained acidosis similar to that seen in Na^+-free solution. Figure 6 compares the effect of Na_o^+ removal with EIPA addition. Note that in both cases pH_i acidified and that removing Na_o^+, after $\approx$ 5 min treatment with EIPA, produced no *additional* acid loading.

The above results indicate that in HEPES-buffered media, pH_i is maintained at an alkaline value by means of acid extrusion on Na^+–H^+ exchange. It is pertinent that EIPA application acidified pH_i at an initial rate similar to that seen in a Na^+-free medium (Fig. 6). In four such experiments the mean rates of initial acidification were $0·247 \pm 0·051$ pH units min^{-1} (mean $\pm$ S.E.M.) in sodium-free media and $0·201 \pm 0·043$ pH units min^{-1} in the presence of 100–150 μM-EIPA. These values were

not significantly different (paired t test, $P > 0{\cdot}05$). While both procedures should maximally inhibit acid efflux on Na^+–H^+ exchange (thus exposing any background H^+ leak or H^+ generation within the cell), Na^+-free treatment might be expected to *reverse* Na^+–H^+ exchange (i.e. promoting H^+ influx/Na^+ efflux) thus *accelerating* the

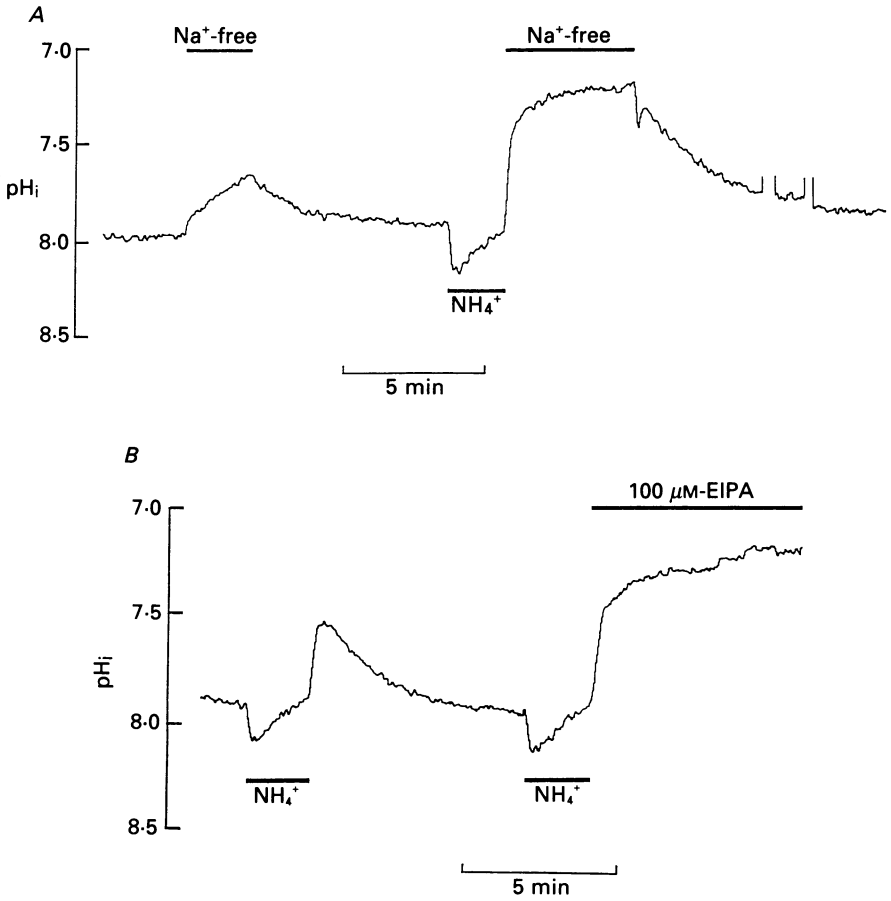

Fig. 5. *A*, effects of removal of external sodium on steady-state pH_i and on pH_i recovery following an induced acid load (removal of 10 mM-NH_4Cl). Single type I cell, bathed in HEPES-buffered Tyrode solution. Sodium was replaced with an equimolar quantity of *N*-methyl-D-glucamine. Similar results were obtained in six other cells. *B*, effects of 100 μM-EIPA on pH_i recovery from an induced acid load (removal of 10 mM-NH_4Cl); clump of type I cells in HEPES-buffered Tyrode solution. Similar results were obtained in ten other experiments.

fall in pH_i. The lack of a significant acceleration of acidosis in Na^+-free solution therefore indicates that backflux of acid equivalents through the exchanger in Na^+-free solution must be a very low fraction of the background acid loading rate. Therefore measurement of $d(pH_i)/dt$ in either EIPA or Na^+-free solution should give a reasonable estimate of the general background acid loading rate in this cell type (see below).

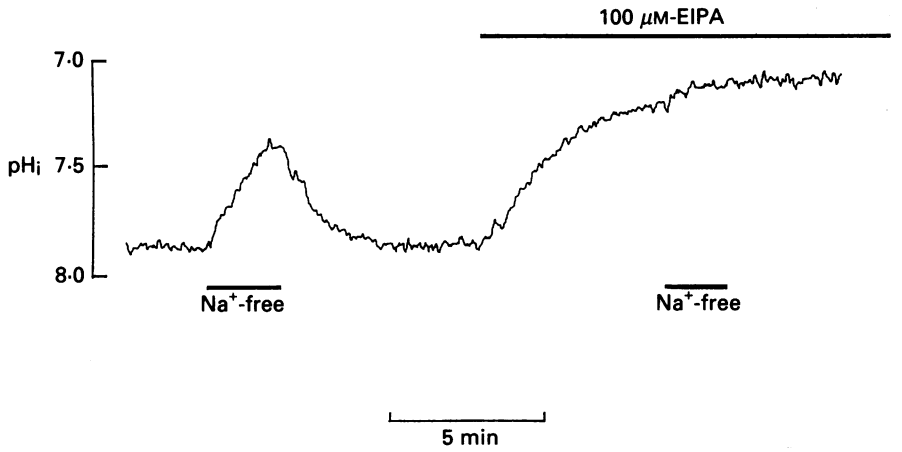

Fig. 6. Effects of Na^+-free Tyrode solution (NMG) and 100 μM-EIPA on pH_i in a type I cell bathed in HEPES-buffered Tyrode solution.

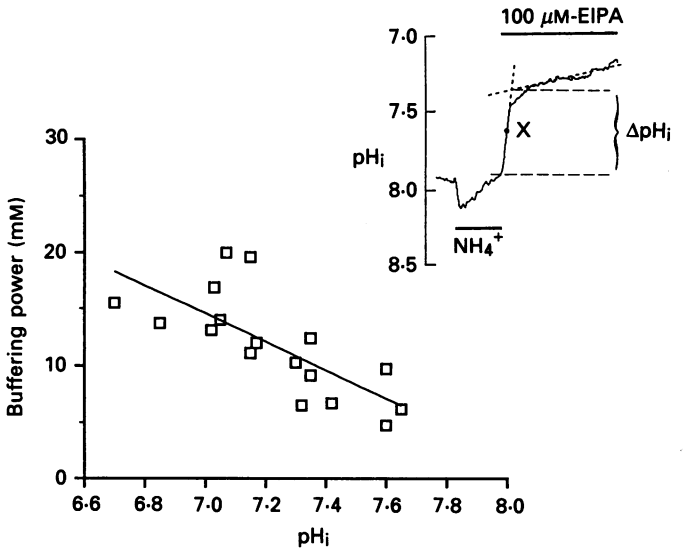

Fig. 7. Dependence of intrinsic intracellular buffering power (β) on pH_i (17 measurements from 12 cells). β was determined in HEPES-buffered Tyrode solution by the injection of intracellular acid using the ammonium pre-pulse technique. During determination of β acid extrusion was inhibited by removal of external sodium or addition of 100 μM-EIPA (see inset, taken from Fig. 5*B*). The concentration of acid injected was determined as described in Methods and the consequent fall of pH_i (ΔpH_i) was measured as shown in inset. For convenience, the estimate of β was related graphically to the value of pH_i measured at 50% acid loading (i.e. point X in the inset). Line fitted by least-squares linear regression: $\beta = 101{\cdot}5 - 12{\cdot}4\ pH_i$ (correlation coefficient = 0·72).

Intrinsic intracellular buffering power

In order to quantify the changes of pH_i in terms of acid equivalent movements, it is necessary to know the intrinsic (non-CO_2) intracellular buffering power, β (acid equivalent fluxes, J_H, can then be estimated as $\beta\ d(pH_i)/dt$).

We have estimated β (see Methods) after inhibiting Na^+–H^+ exchange (Na^+-free solution or EIPA addition) and then performing an NH_4^+ pre-pulse to produce a fall in pH_i. By inhibiting the exchanger we limit ourselves to observations of intracellular buffering exclusive of contributions from transmembrane acid efflux (Bountra *et al.*

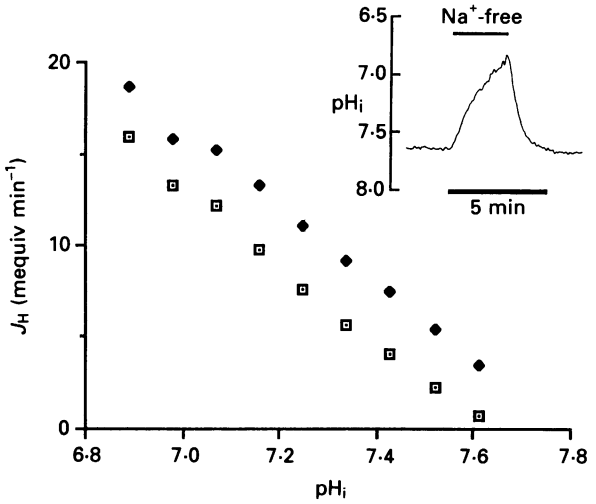

Fig. 8. The pH_i dependence of Na^+–H^+ exchange. Graph shows acid efflux *versus* pH_i. Acid efflux was measured following re-addition of external sodium (see inset). Value of β used in calculation of fluxes was determined from the equation given in Fig. 7. ⊡, net acid efflux; ◆, unidirectional acid efflux (i.e. net acid efflux minus background acid loading; see text for further details).

1990). Figure 7, which pools data from seventeen cells, shows a plot of β *versus* pH_i. Two features merit attention; (i) the overall value of β is quite low, e.g. at pH_i 7·5, β is $\approx$ 8 mM, and (ii) although there is some scatter, the value of β tends to *increase* as pH_i falls, roughly doubling for a 1·0 unit decrease in pH_i. This pH_i dependence of β is comparable to that observed recently in the cardiac Purkinje fibre (Vaughan-Jones & Wu, 1990*a*) and the renal mesangial cell (Boyarsky, Ganz, Sterzel & Boron, 1988*a*).

pH_i dependence of Na^+–H^+ exchange

Figure 8 plots net acid efflux (J_H, expressed as $-\beta \mathrm{d}pH_i/\mathrm{d}t$) as a function of pH_i, data being obtained from the Na^+-dependent pH_i recovery shown in the inset. The pH_i-dependent value of β used in the calculation of acid efflux was estimated from the line of best fit (linear regression) to the data shown in Fig. 7 (see legend to Fig. 7). Note that, in Fig. 8, net acid efflux (open symbols) increases nearly 15-fold as pH_i falls from 7·6 to 6·9. This net efflux, however, represents Na^+-dependent acid efflux *in excess* of any background acid loading that occurs in the absence of Na^+–H^+ exchange. The background loading is significant, as evidenced in Figs 5 and 6 (observe the acid loading in either Na^+-free solution or EIPA; see above). An advantage of acid loading the cell by temporary Na_o^+ removal is that both background acid loading ($\beta \mathrm{d}pH_i/\mathrm{d}t$) and net acid efflux may be determined in the same cell and over the same range of pH_i values. If this background loading rate is

added to the net acid efflux rate determined in Fig. 8, one should approximate more realistically the *total* acid efflux on Na^+–H^+ exchange. When this is done, the relationship shown by the filled symbols in Fig. 8 is obtained. Observe that (i) this relationship bears a similar slope to the uncorrected data but that it is displaced

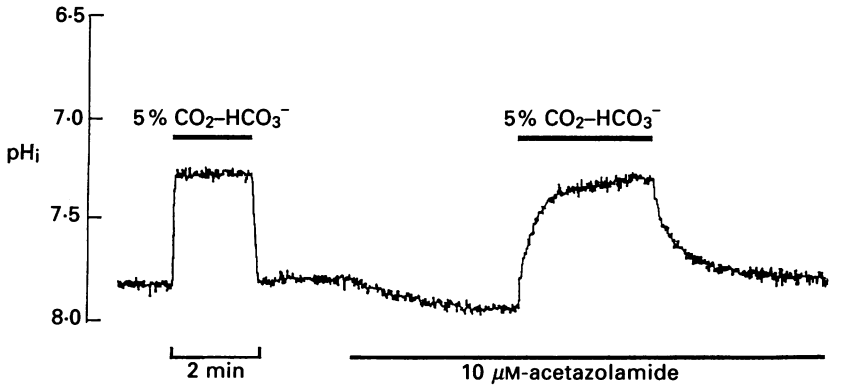

Fig. 9. Effects of 10 μM-acetazolamide on CO_2-induced changes in pH_i. A type I cell initially in HEPES-buffered media, pH 7·4, was briefly exposed to a 5% CO_2–HCO_3^--buffered Tyrode solution (also $pH_o = 7{\cdot}4$) in the absence and presence of the membrane permeant carbonic anhydrase inhibitor, acetazolamide. Note the slowing of the CO_2-induced acidosis in acetazolamide.

significantly to the right along the abscissa (i.e. towards more alkaline values of pH_i) and (ii) unidirectional acid efflux now increases 5-fold (rather than 15-fold) upon reduction of pH_i from 7·6 to 6·9. This demonstrates the importance of including background acid loading rates when analysing the pH_i dependence of acid extrusion in a given cell, as shown recently by Vaughan-Jones & Wu (1990*b*); results similar to Fig. 8 were obtained in four other cells. In all experiments the relationship between unidirectional acid efflux and pH_i was found to be approximately linear over a range of pH_i from 7·9 to 6·83 with no apparent indication of saturation. It is therefore apparent that pH_i must be manipulated to values substantially lower than those used in the present study (i.e. $pH_i < 6{\cdot}83$) in order to characterize fully the activation curve of Na^+–H^+ exchange in this tissue.

pH_i regulation in the presence of CO_2–HCO_3^-

Intracellular carbonic anhydrase

Figure 9 shows the effects on pH_i of switching to a 5% CO_2–HCO_3^--buffered medium (same pH_o, 7·40). This cell was initially rather alkaline (in HEPES solution) so that a rapid and reversible fall of pH_i was observed upon adding CO_2 ($t_{0.5} < 5$ s; this is probably limited by the bath exchange time which has a $t_{0.5}$ of ≈ 3 s). The second part of the experiment shows the effect of adding acetazolamide (10 μM), a membrane-permeant carbonic anhydrase inhibitor. Resting pH_i (in HEPES medium) became even more alkaline in acetazolamide. Subsequent application of CO_2–HCO_3^- solution now produced a similar, but much slower fall in pH_i. This slower fall was 50% complete in about 12 s, a time course which is similar to that predicted from the

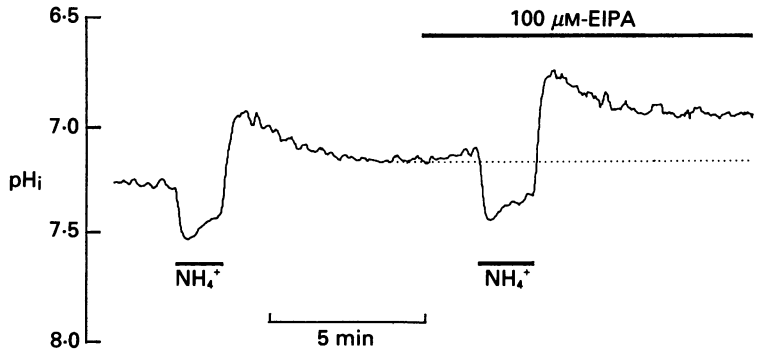

Fig. 10. Effects of 100 μM-EIPA on pH_i recovery from an induced intracellular acid load (removal 10 mM-NH_4Cl) in a type I cell bathed in a 5% CO_2–HCO_3^--buffered Tyrode solution.

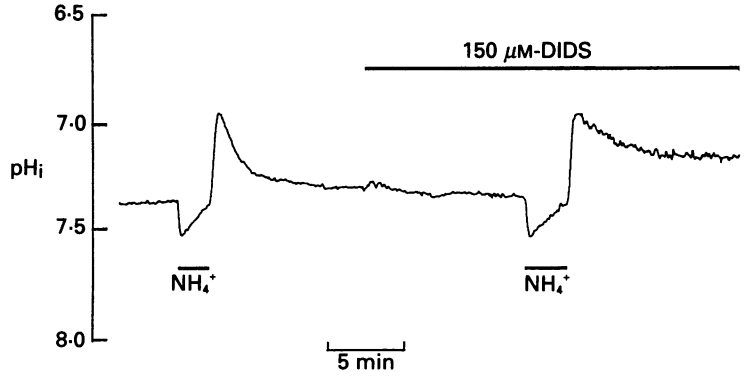

Fig. 11. Partial inhibition of pH_i recovery from an induced intracellular acid load by 150 μM-DIDS in a clump of type I cells bathed in 5% CO_2–HCO_3^--buffered Tyrode solution.

non-catalysed hydration of CO_2. We therefore conclude that intracellular carbonic anhydrase in the type I cell normally catalyses CO_2 hydration, thus producing very rapid pH_i responses to extracellular changes of P_{CO_2}. The observations also indicate that CO_2 membrane permeation was unlikely to have been rate limiting during our pH_i measurements.

Na^+–HCO_3^--dependent pH_i recovery

Figure 10 shows that EIPA (100 μM) does not completely inhibit pH_i recovery in a CO_2–HCO_3^--buffered medium. Similar results were obtained in two experiments with amiloride and in three out of four experiments using EIPA. This is in marked contrast to the effects of EIPA in HEPES medium (Fig. 5*B*) where complete inhibition was seen. Thus, in the presence of CO_2, Na^+–H^+ exchange is probably not the only mechanism for pH_i recovery following internal acidosis. This conclusion is reinforced by the experiment shown in Fig. 11. Here, pH_i recovery (following an NH_4^+ pre-pulse) was slowed, but not inhibited entirely, by the stilbene, DIDS (150 μM), a known inhibitor of transmembrane bicarbonate movement (Thomas, 1984).

The data shown in Fig. 12*A* confirms that, in the presence of CO_2–HCO_3^-, mechanisms other than Na^+–H^+ exchange assist pH_i recovery. As before (Fig. 5*A*), pH_i recovery from an acid load was largely inhibited in Na^+-free solution indicating a Na^+ requirement for net acid extrusion. Re-admitting Na^+ in the presence of EIPA

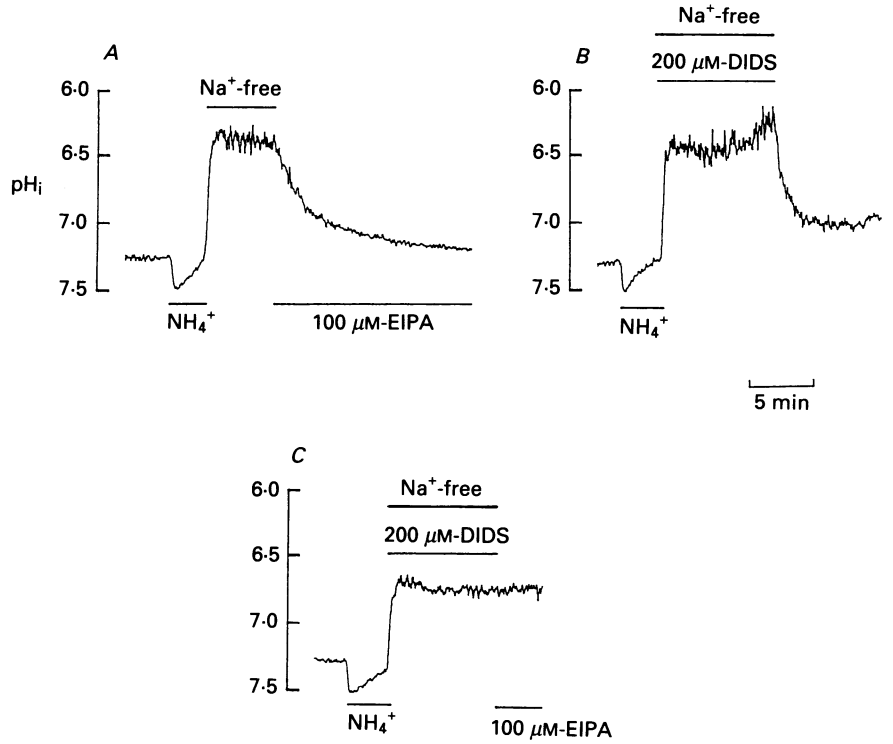

Fig. 12. Effects of Na^+-free (NMG) solution, EIPA and DIDS on pH_i recovery following induced acid load in CO_2–HCO_3^--buffered Tyrode solution. All cells were first exposed to 10 mM-NH_4Cl and then transferred to a Na^+-free Tyrode solution. *A*, sodium reintroduced with concomitant addition of 100 μM-EIPA. Pair of type I cells. *B*, single type I cell pre-treated with 200 μM-DIDS in Na^+-free Tyrode solution before reintroduction of external sodium. *C*, pair of type I cells pre-treated with 200 μM-DIDS in Na^+-free Tyrode solution before reintroduction of external sodium with concomitant addition of 100 μM-EIPA.

(100 μM) then produced a clear recovery of pH_i from 6·5 to 7·25 (Fig. 12*A*). Figure 12*B* shows that re-admitting Na_o^+ following pre-treatment with 200 μM-DIDS also promoted pH_i recovery. Re-admitting Na_o^+, however, in a cell pre-treated with DIDS (200 μM) *and* in the presence of 100 μM-EIPA inhibited pH_i recovery entirely (Fig. 12*C*), similar results were obtained in three other experiments. These data therefore indicate that, in addition to Na^+–H^+ exchange (EIPA inhibited), a sodium- and bicarbonate-dependent mechanism (DIDS inhibited) can produce prompt pH_i recovery from an intracellular acid load.

Na^+-independent anion exchange

Although the above data indicate clearly the Na^+ requirement of HCO_3^--dependent pH_i recovery from an acid load, they do not exclude the possible, parallel existence of a Na^+-independent Cl^-–HCO_3^- exchange mechanism, as has been described in the

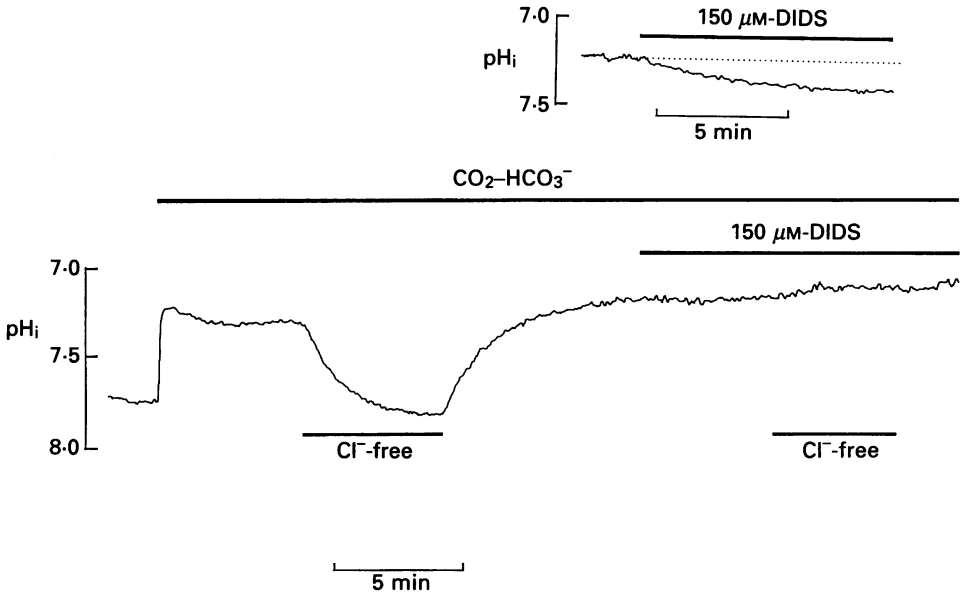

Fig. 13. Main figure, effect on pH_i of Cl^- removal (Cl^- replaced with glucuronate) in the absence and presence of 150 μM-DIDS in a single type I cell in 5% CO_2–HCO_3^--buffered Tyrode solution. Inset, effect of 150 μM-DIDS on steady-state pH_i in a clump of type I cells in a 5% CO_2–HCO_3^--buffered Tyrode solution.

cardiac Purkinje fibre (Vaughan-Jones, 1982), the renal mesangial cell (Boyarsky, Ganz, Sterzel & Boron, 1988*b*) and other cells (Tonnessen, Ludt, Sandvig & Olsnes, 1987; Putnam, 1990). This latter system, while contributing little to acidic pH_i recovery, contributes significantly to pH_i recovery from an *alkaline* load and may contribute to background acid influx in the steady state.

Note that in Fig. 11 and in the inset to Fig. 13, application of DIDS (a known inhibitor of anion exchange) produced a small *increase* of resting pH_i (by 0·2 units in the inset to Fig. 13). This was not observed in all cells (see e.g. the main part of Fig. 13), but it was sufficiently common (six out of nine experiments) to lead us to suspect that a DIDS-sensitive anion exchanger, producing a resting acid equivalent *influx* (Vaughan-Jones, 1982) may also be present in the type I cell. Thus inhibition of this acid influx by DIDS could promote a rise in resting pH_i.

The existence of Cl^-–HCO_3^- exchange can be demonstrated by looking at Cl^--dependent HCO_3^- movement. In other tissues Cl_o^- removal has been shown to promote Cl^- efflux/HCO_3^- influx via the exchanger, thus raising pH_i. This effect is known to be both inhibitable by stilbene derivatives (Vaughan-Jones, 1979*b*) and independent of external sodium (Boyarsky *et al.* 1988*b*). Figure 13 shows that

exposure of a type I cell to Cl^--free solution (replaced by glucuronate) produced a reversible alkalosis which was DIDS inhibited ($n = 4$). An alkalosis upon Cl^--removal was also observed in Na^+-free solution (Fig. 14, $n = 6$), thus excluding contributions to alkalosis from any Na^+-dependent mechanisms. We also observed alkalosis in Cl^--

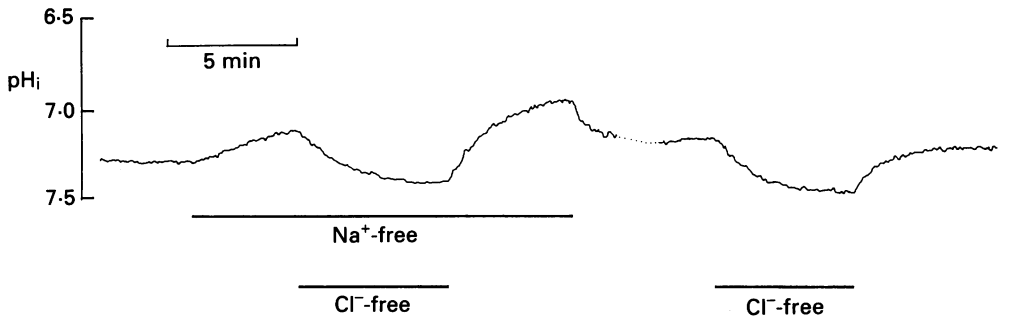

Fig. 14. Effect of Cl^- removal (Cl^- replaced with glucuronate) in the presence and absence of external Na^+ (NMG substituted) on pH_i in a single type I cell in a 5% CO_2–HCO_3^--buffered Tyrode solution. Break in trace (dotted line) marks where photomultiplier tubes were switched off while adjustments were made to the solution flow.

free HEPES media (nominally CO_2 free, not shown). We conclude, therefore, that a Na^+-independent, DIDS-sensitive Cl^-–HCO_3^- exchanger can influence pH_i in the type I cell. The small rise of normal resting pH_i produced by addition of DIDS to type I cells perfused with CO_2–HCO_3^- solution suggests that this anion exchange mechanism may resemble that seen in other cells where Na^+-independent Cl^-–HCO_3^- exchange mediates net acid *influx*. Note however that, given the presence of two opposing DIDS-sensitive acid flux mechanisms in the type I cell (i.e. Cl^-–HCO_3^- exchange and a Na^+–HCO_3^--dependent acid efflux), the effect on steady-state pH_i of blocking *both* with DIDS will depend upon which mechanism was initially the more active.

The persistence of some Cl_o^--dependent pH_i changes in nominally CO_2-free solution may reflect the presence of residual levels of intracellular HCO_3^- (possibly generated by hydration of metabolically produced CO_2), a finding reported for other cells (Roos & Boron, 1981; Vaughan-Jones, 1986). In this context, it is notable that the application of acetazolamide increases pH_i in HEPES-buffered media (Fig. 9). This may result from the inhibition of hydration of metabolically derived CO_2.

Physiological importance of HCO_3^--dependent transporters

The presence of at least two HCO_3^--dependent acid equivalent transporters in the type I cell (i.e. a Na^+–HCO_3^--dependent mechanism and Cl^-–HCO_3^- exchange), complicates analysis of the pH_i dependence of acid movement in CO_2–HCO_3^- solution. Figure 11, however, indicates that a DIDS-sensitive mechanism accounts for about 50% of the rate of pH_i recovery from an acid load, when measured at a pH_i of 7·0 (a similar result was obtained in two other cases). In cardiac muscle (Vaughan-Jones, 1982), cultured Vero cells (Tonnessen *et al.* 1987) and renal mesangial cells

(Boyarsky *et al.* 1988*b*), Cl^-–HCO_3^- exchange contributes little if at all to pH_i recovery from an acid load. If the same applied to type I cells then the Na^+–HCO_3^--dependent mechanism would be responsible for the above 50% contribution towards acid extrusion.

DISCUSSION

The present work shows that intracellular pH in the principal arterial pH receptor (i.e. the type I cell) is regulated efficiently. Under physiological conditions (i.e. with CO_2–HCO_3^-) pH_i is about 7·3 and following an intracellular acid load, it returns to control levels promptly by means of transmembrane acid equivalent transport. At least three separate mechanisms have been identified that can assist in the regulation of pH_i. These are illustrated diagrammatically in Fig. 15 and are (i) Na^+–H^+ exchange (ii) Na^+–HCO_3^--dependent acid transport and (iii) Cl^-–HCO_3^- exchange. The first two mechanisms account for net acid efflux, thus promoting pH_i recovery from an acidosis. The role of the third mechanism (Cl^-–HCO_3^- exchange) has yet to be elucidated although, in the steady state it seems to mediate a small HCO_3^- efflux (equivalent, in an open CO_2 system, to an acid *influx*). Lastly, the presence of intracellular carbonic anhydrase greatly assists the speed of all pH_i changes induced by changes of P_{CO_2}.

Resting pH_i in the type I cell

We find pH_i in the presence of CO_2–HCO_3^- to be considerably less acidic (pH_i 7·3) than reported previously for the type I cell (pH_i 6·9: Biscoe, Duchen, Eisner, O'Neil & Valdeolmillos, 1989, using an intracellular fluoroprobe, BCECF; pH_i 6·7: He *et al.* 1988, using an intracellular pH microelectrode). Indeed, these previous reports were both determined in the *absence* of CO_2, conditions where we find pH_i to be even more *alkaline* (i.e. 7·8). The reason for these apparent differences in pH_i is not known. We would point out that our own pH_i recordings have been calibrated by two *independent* methods which are in good agreement. Furthermore, although pH_i can be unusually alkaline with HEPES media, it always reverts to a more typical value of $\approx$ 7·3 with CO_2–HCO_3^- media. Low values for pH_i, obtained with ion-selective microelectrodes (He *et al.* 1988), could be due to inadequate membrane sealing around the microelectrode during cell impalement, causing an inward acid leak (so called 'acid-injury', see Aickin & Thomas, 1975). This would not, however, account for the low pH_i seen with intracellular BCECF (Biscoe *et al.* 1989). It should be noted, however, that this latter value is for a single recording of pH_i.

An alternative explanation for different pH_i values is that they are related to the cell storage conditions. For example, Biscoe *et al.* (1989) kept type I cells before use at 3–4 °C in HEPES media whereas we have used culture media buffered with 5% CO_2–HCO_3^- at 37 °C. Green, Frelin, Vigne & Lazdunski (1986) have reported that the use of different culture media can alter the apparent H_i^+ affinity of Na^+–H^+ exchange in cultured chick cardiac myocytes thus affecting resting pH_i. We report here (Fig. 4) that overnight storage plus subsequent perfusion in HEPES media at 37 °C produces a less alkaline pH_i ($pH_i \approx 7{\cdot}3$ instead of 7·8 in those cells stored in CO_2–HCO_3^- media and then perfused in HEPES media). Since biochemical

modulation of the pH_i dependence of Na^+–H^+ exchange, either by intracellular or extracellular factors, is a common feature in cells (see Moolenaar, 1986) it is possible that switching from CO_2–HCO_3^- to HEPES-buffered solution might also, in some way, slowly reset the activity of Na^+–H^+ exchange in the type I cell. This might then

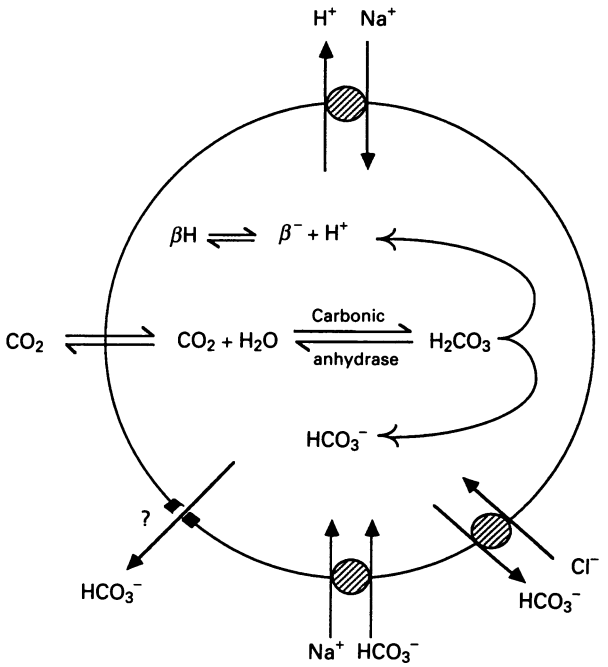

Fig. 15. Model of pH_i regulation in the type I cell of the carotid body. The three identified acid equivalent carriers are indicated (note that the full ionic dependence of the Na^+–HCO_3^- system has not yet been determined). Also shown is the putative HCO_3^- channel (see Discussion), and the intracellular buffers consisting of intrinsic buffering (β) plus the open system for CO_2–HCO_3^-.

explain why short-term storage (up to 60 min) in HEPES gave a high pH_i of 7·8, whereas long-term storage (24 h) gave a much lower pH_i of 7·3. For this to occur, the set point of Na^+–H^+ exchange would need to have been readjusted to a more acidic pH_i. Alternatively, long-term storage in HEPES media might increase the background rate of acid loading, caused by unidentified H^+ influx mechanisms or by internal metabolic processes. If the properties of Na^+–H^+ exchange remained unchanged, this would also result in a fall of resting pH_i.

Whatever the reason for variability of pH_i with non-CO_2-buffered media, we find that pH_i is always stabilized close to 7·3 in CO_2–HCO_3^-, ruling out general run down of cells as the explanation of pH_i differences. Thus, when examining physiological responses in type I cells, we recommend the use of CO_2–HCO_3^- media in order to eliminate possible abnormalities of pH_i.

Intrinsic buffering power in the type I cell

Although the non-CO_2 buffering power (β) estimated in the type I cell varied with pH_i it was always relatively low (6–20 mM in the pH_i range, 7·8–6·8). The low value for β is similar to that now reported for a variety of other cell types (Boyarsky *et al.* 1988*a*; Szatkowski & Thomas, 1989; Bountra *et al.* 1990; Putnam & Grubbs, 1990). The increase of β with a fall in pH_i also seems to be a common feature, probably reflecting a low mean pK_a for intracellular buffers (Boyarksy *et al.* 1988*a*: Szatkowski & Thomas, 1989; Wenzl, Sjaastad, Wientraub & Machen, 1989; Vaughan-Jones & Wu, 1990*a*). We conclude, therefore, that the intrinsic H^+ buffering capacity of type I cells is not specialized with respect to the cell's function as an H^+ ion chemoreceptor. The rapid catalysis of CO_2-induced pH_i changes by carbonic anhydrase ensures *fast* H^+ buffering in the type I cell, but this phenomenon is, again, not unique (cf. Thomas, 1984). Finally, the relatively low values of β determined in the present work suggest that the intracellular fluoroprobe itself, contributes little to H^+ buffering and so its presence is unlikely to have significantly slowed or attenuated the pH_i responses. Note also that the control pH_i changes recorded in Fig. 9 occurred with a $t_{0.5}$ of ≈ 3 s and that this time course was likely to have been rate limited by the bath exchange time rather than by the kinetics of the fluoroprobe, or of carbonic anhydrase.

Intracellular pH regulation in the type I cell

An important finding is that pH_i in the type I cell is efficiently regulated so that displacements of pH_i in the acid direction are readily removed within 5 min or so. Regulation of pH_i from an intracellular *alkaline* load has not been tested but the presence of a resting acid *influx* via Cl^-–HCO_3^- exchange is consistent with the existence of such a mechanism (Vaughan-Jones, 1982; Tonnessen *et al.* 1987).

Of the elements responsible for pH_i regulation, Na^+–H^+ and Cl^-–HCO_3^- exchange have been demonstrated commonly in other cells. We have not identified the full ionic dependencies of the additional Na^+–HCO_3^--dependent mechanism in the type I cell but it could be either a Na^+-dependent Cl^-–HCO_3^- exchanger akin to that described in squid axon, snail neurone, and various other cells (Thomas, 1977; Boron & Russell, 1983) or a Na^+–HCO_3^- co-transport mechanism as proposed in glia (Deitmer & Schlue, 1989), smooth muscle (Boyarsky *et al.* 1988*b*) and renal epithelia (Boron, 1986). The apparent combination of so many acid equivalent transporters in a cell membrane is no longer considered to be unusual. One must consider, however, the possibility that the three identified mechanisms in the type I cell are merely partial reactions of a single multi-ion transporter. This seems unlikely, since in other cells the primary sequences of specifically identified Na^+–H^+ and Cl^-–HCO_3^- exchange proteins show many areas of non-homology (Sardet, Franchi & Pouyssegur, 1989). At present therefore we assume that the three pathways identified in the type I cell are likely to be independent proteins. We should, however, consider the possibility that part of the HCO_3^--dependent acid efflux pathway may actually be a HCO_3^- ion channel rather than a carrier. Such a channel has been identified in the type I cell (Stea & Nurse, 1989); it is an anion channel with (roughly) equal selectivity for Cl^- and HCO_3^- ions. Any HCO_3^- influx via this channel would

contribute to pH_i recovery. This would occur, however, only if the membrane potential (E_m) were positive to E_{HCO_3} (the equilibrium potential for HCO_3^- ions), i.e. (assuming an open CO_2 system) at a pH_i of 7·1–7·2, if E_m were *positive* to about −10 mV. This is an unlikely possibility since E_m in the type I cell has been estimated to be about −50 mV (O'Donnell, Ashcroft, Brown & Nye, 1989). Thus, as illustrated in Fig. 15, passive HCO_3^- movement through channels would be outward not inward. Consequently it is unlikely that the Na^+–HCO_3^--dependent acid efflux is due partly to passive HCO_3^- influx through anion channels. We do not rule out the existence of a HCO_3^- channel, but it would seem not to contribute to pH_i recovery. Rather, its existence would *impede* such recovery by promoting passive HCO_3^- *efflux*.

Role of pH_i regulation in the chemotransduction process

Hydrogen ion chemoreception by the type I cell is suggested to occur via changes of pH_i rather than pH_o (Hanson, Nye & Torrance, 1981) although earlier hypotheses outlined a dominant role for pH_o in the carotid body (Torrance, 1977). Given the multiplicity of H^+-regulating mechanisms in the type I cell (Fig. 15), this H^+ chemoreceptor would seem ideally suited as a system for maintaining a constant pH_i. It is intriguing, therefore, that this is not actually the case, as we reported recently (Buckler, Nye, Peers, Vaughan-Jones, 1990*b*). Following changes of pH_o, pH_i in the type I cell also changes by a near-equivalent amount. A more complete analysis of the relationship between pH_i and pH_o in the type I cell will be presented in a future paper (K. J. Buckler, P. C. G. Nye, C. Peers and R. D. Vaughan-Jones, in preparation). The demonstration of an efficient pH_i regulation system in the type I cell should not therefore be considered to contradict the possibility of an intracellular site for H^+ chemotransduction. Furthermore, the slowing of CO_2-induced pH_i changes in the type I cell by acetazolamide parallels its slowing of the *in vivo* response of the carotid body to a respiratory acidosis (McCloskey, 1968). This is consistent with changes of pH_i mediating the chemotransduction.

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